ELSEVIER

Comparative Biochemistry and Physiology, Part C 140 (2005) 151 – 164

CBP

www.elsevier.com/locate/cbpc

Review

Polyamine transport in parasites: A potential target for new antiparasitic drug development

Rosa María Reguera[a], Babu L. Tekwani[b], Rafael Balaña-Fouce[a,*]

[a]*Department of Pharmacology and Toxicology (INTOXCAL), University of Leon, Campus de Vegazana (s/n) 24071 Leon, Spain*
[b]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA*

Received 27 September 2004; received in revised form 7 February 2005; accepted 8 February 2005

Abstract

The metabolism of the naturally occurring polyamines—putrescine, spermidine and spermine—is a highly integrated system involving biosynthesis, uptake, degradation and interconversion. Metabolic differences in polyamine metabolism have long been considered to be a potential target to arrest proliferative processes ranging from cancer to microbial and parasitic diseases. Despite the early success of polyamine inhibitors such as alpha-difluoromethylornithine (DFMO) in treating the latter stages of African sleeping sickness, in which the central nervous system is affected, they proved to be ineffective in checking other major diseases caused by parasitic protozoa, such as Chagas' disease, leishmaniasis or malaria. In the use and design of new polyamine-based inhibitors, account must be taken of the presence of up-regulated polyamine transporters in the plasma membrane of the infectious agent that are able to circumvent the effect of the drug by providing the parasite with polyamines from the host. This review contains information on the polyamine requirements and molecular, biochemical and genetic characterization of different transport mechanisms in the parasitic agents responsible for a number of the deadly diseases that afflict underdeveloped and developing countries.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Ornithine decarboxylase; Polyamine inhibitors; Sleeping sickness; Chagas disease; *Trypanosoma*; *Leishmania*; *Trichomonas*; Adenosyl methionine

Contents

1. Introduction 152
2. "De novo" polyamine synthesis 152
3. Polyamine transport systems 155
   3.1. Polyamine carriers in bacteria and yeast 155
   3.2. Polyamine transport in mammalian cells 156
4. Polyamine uptake in parasites 157
5. Regulation of polyamine uptake 158
6. Inhibition of polyamine uptake 160

Abbreviations: Ornithine decarboxylase, (ODC); *S*-adenosylmethionine decarboxylase, (SAMDC); α-difluoromethylornithine, (DFMO); Arginine decarboxylase, (ADC); Spermidine/spermine N^1-acetyltransferase, (SSAT); *S*-adenosylmethionine, (AdoMet); *S*-adenosylhomocysteine, (AdoHcy); ATP binding cassette, (ABC); ODC antizyme, (AZ); Polyamine deficient chow, (PDC).

* Corresponding author. Tel.: +34 987 291590; fax: +34 987 291252.
E-mail address: dftrbf@unileon.es (R. Balaña-Fouce).

1532-0456/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cca.2005.02.006

7. Dietary polyamine deprivation . . . 160
8. Concluding remarks . . . 161
Acknowledgments . . . 161
References . . . 161

1. Introduction

Tropical diseases caused by parasitic protozoa are among humanity's costliest banes both for the high mortality rates involved and the economic loss resulting from morbidity. Malaria, African and American trypanosomiasis and leishmaniasis, among others, are diseases that have an enormous impact on global health. Every year, the etiological agents involved infect several hundred million people and take millions of lives. The social and economic costs of these diseases are also very high. The social consequences of tropical diseases impede development in various ways, lowering fertility rates, population growth, savings and investment and productivity and raising absenteeism, premature mortality and medical costs. The factors contributing to the growth in the incidence of tropical diseases include the appearance of drug resistant strains of parasites, insecticide-resistant vectors, global warming, growing populations in underdeveloped and developing endemic countries and travel to these countries. The effects of tropical diseases now threaten developed countries as opportunistic infections in immuno-compromised patients, increasing the mortality rate of AIDS.

2. "De novo" polyamine synthesis

Polyamine metabolism (Fig. 1) was one of the promising pathways explored in the early eighties to combat several diseases, including illness caused by unicellular parasites (Bacchi et al., 1980). Polyamines—putrescine, spermidine and spermine—are naturally occurring polycationic biomolecules, essential for cellular growth and proliferation in all living organisms (see reviews by: Muller et al., 2001; Bacchi and Yarlett, 2002; Heby et al., 2003; Wallace et al., 2003). Under physiological conditions, the role of polyamines is attributed, in both parasite and host, to the readily protonated amino/imino groups to which they owe their cationic strength at intracellular pH. This property enables polyamines to interact spontaneously with long anionic macromolecules, such as nucleic acids, proteins and lipids. Many cellular processes are polyamine-dependent. Intracellular polyamine levels are known to peak during cell proliferation in tumoral or infectious processes, and are a key target in chemotherapeutic processes.

All vertebrates, lower eukaryotes including fungi and most protozoa synthesize polyamines de novo from two amino acid precursors: L-ornithine and L-methionine. Putrescine (1,4-diaminobutane) is enzymatically synthesized from L-ornithine by ornithine decarboxylase (ODC), a developmentally regulated enzyme. Spermidine synthesis, in turn, involves condensation of putrescine with the aminopropyl moiety of decarboxy-*S*-adenosylmethionine, in a reaction mediated by the enzyme spermidine synthase in the absence of ATP. In mammals, spermidine is condensed with a second aminopropyl group by the enzyme spermine synthase to produce spermine. Polyamine levels as well as the activity of their biosynthetic enzymes ODC and *S*-adenosyl methionine decarboxylase (SAMDC) have been found to increase during rapid growth. Therefore, polyamine biosynthesis is a potential biochemical target in the development of anti-parasitic drugs. Many in vivo trials have been conducted with polyamine antimetabolites, the most important of which is α-difluoromethylornithine (DFMO), an irreversible inhibitor of ODC and the first antitrypanosomal compound to be included in the pharmacopoeia for forty years (for a clinical review, see Burri and Brun, 2003).

ODC (EC 4.1.1.17) is the enzyme responsible for putrescine synthesis from L-ornithine. It has been cloned from many sources, including *T. brucei* (Phillips et al., 1987), *L. donovani* (Hanson et al., 1992) and *C. fasciculate* (Svensson et al., 1997). *T. brucei* and *Leishmania* spp. ODCs have a long half-life (more than 6 h), unlike the host protein (less than 30 min) (Phillips et al., 1987; Sánchez et al., 1989; Reguera et al., 1995). A long ODC half-life seems to be one of the requisites for DFMO-sensitivity in parasites (Carrillo et al., 2000). Indeed, thanks to this specific feature, while it is impossible for the parasite to replace the ODC targeted by DFMO, the enzyme can be readily replaced in the host. The carboxy-terminal region of mammalian ODC is crucial to rapid protein turnover. The absence of two PEST motifs responsible for ODC interaction with its antizyme, and the degradation by proteosomes in *T. brucei*, are plausible reasons for this long half-life (Ghoda et al., 1990). However, the existence of a short half-life ODC in *C. fasciculata*, which also lacks the above domain, indicates that other motifs on the protein may be involved in ODC degradation and turnover (Ceriani et al., 1992; Svensson et al., 1997). Moreover, the fact that no ODC antizyme (AZ; see below) has been found in any protozoan or nematode worm suggests the mediation of an alternative AZ-independent system in the degradation of the enzyme (Persson et al., 2004).

Unlike most trypanosomatids, *T. cruzi*, the etiological agent of Chagas' disease in South America, is naturally auxotrophic for putrescine, as it lacks the gene encoding for ODC (Carrillo et al., 1999). This makes *T. cruzi* dependent

upon arginine and polyamine uptake for growth and survival, rendering DFMO useless in the treatment of this disease (Schwarcz de Tarlovsky, 1993). Some authors have suggested that putrescine biosynthesis is dependent on the decarboxylation of L-arginine via arginine decarboxylase (ADC) with the conversion of arginine to agmatine, which is further metabolized to putrescine and spermidine (Hernández and Schwarcz de Tarlowsky, 1999). These results confirmed earlier studies conducted with the ADC inhibitor α-difluoromethylarginine (DFMA), in which arginine-derived polyamines were shown to be essential for parasite proliferation and infection in mammalian cells (Majumder et al., 1992; Yakubu et al., 1992).

In *T. cruzi* epimastigotes, extensive arginine uptake may play a dual role, not only providing a potential polyamine precursor but also acting as a defense mechanism, since it

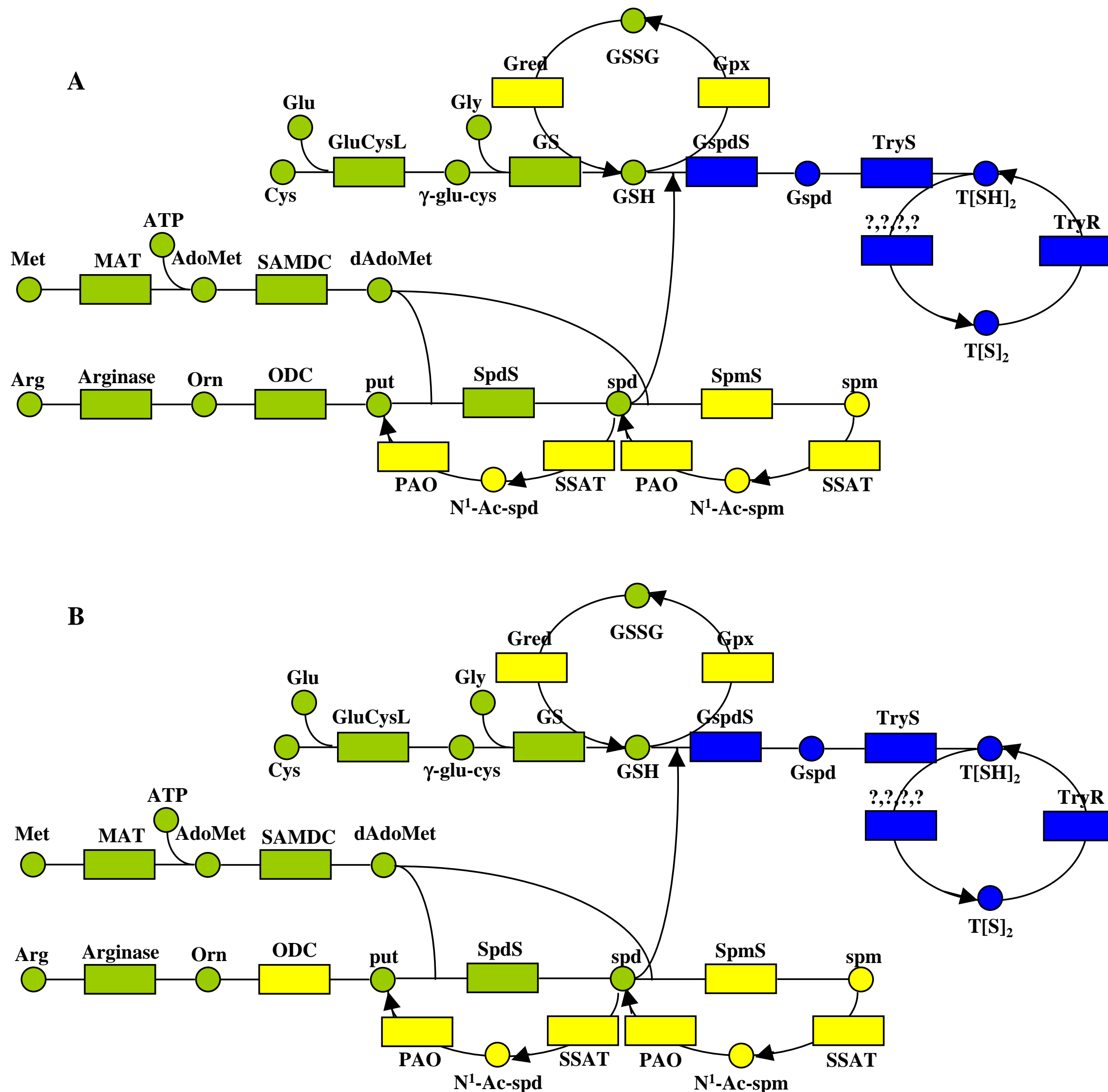

Fig. 1. Schematic representation of polyamine and thiol-redox metabolism in different parasites and their hosts. African trypanosomes, *Leishmania* spp (A) and *T. cruzi* (B) combine both pathways, unlike the microaerophile *Trichomonas vaginalis* (C) which lacks glutathione (Muller et al., 2003) and the Apicomplexan *Plasmodium falciparum* (D). Enzymes and metabolites common for parasites and mammals are colored in green, whereas the specific parasite paths are in blue and host characteristics are in yellow (recommended by Fairlamb, 2002). Abbreviations are as follows: intermediaries: put, putrescine; spd, spermidine; spm, spermine; AdoMet, *S*-adenosylmethionine; dAdoMet, decarboxylated *S*-adenosylmethionine; GSH, reduced glutathione; GSSG oxidized glutathione; $T[S]_2$ oxidized trypanothione; $T[SH]_2$ reduced trypanothione; Gspd, glutathionyl-spermidine; N^1-Ac-spd, N^1acetyl-spermidine; N^1-Ac-spm, N^1acetyl-spermine. Enzymes and EC number: MAT, methionine adenosyltransferase (EC 2.5.1.6); arginase (EC 3.5.3.1); ODC, ornithine decarboxylase (EC 4.1.1.17); SAMDC, *S*-adenosylmethionine decarboxylase (EC 4.1.1.50); SpdS, spermidine synthase (EC 2.5.1.16); SpmS, spermine synthase (EC 2.5.1.22); SSAT, spermidine/spermine-N^1-acetyltransferase (EC 2.3.1.57); PAO, polyamine oxidase (EC 1.5.3.11); GluCysL glutamate–cysteine ligase (EC 6.3.2.2); GS, glutathione synthase (EC 6.3.2.3); Gred glutathione reductase (EC 1.8.1.7); Gpx, glutathione peroxidase (EC 1.11.1.9); glutathionyl-spermidine synthase (EC 6.3.1.8); TryS, trypanothione synthetase (EC 6.3.1.9); TryR, trypanothione reductase (EC 1.6.4.8). (*) Bifunctional ODC/SAMDC is a specific attribute of malaria parasites.

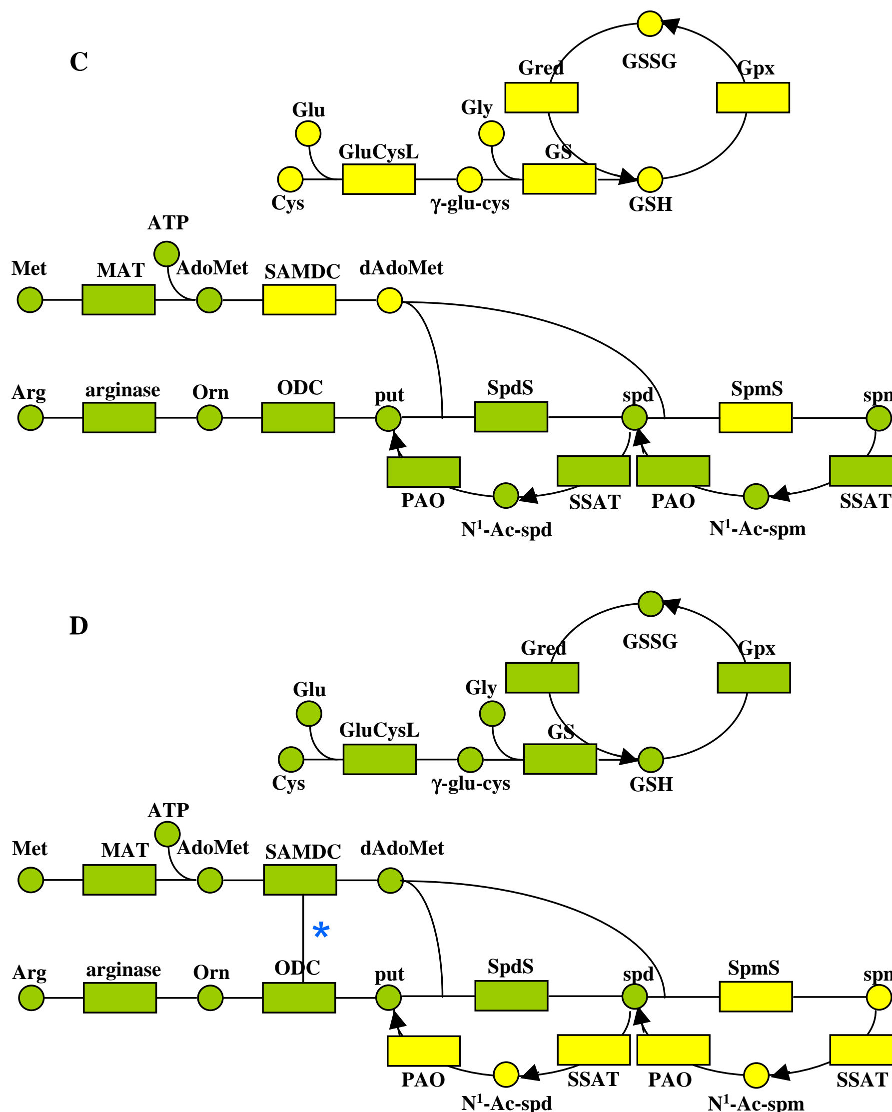

Fig. 1 (*continued*).

entails depletion of the arginine needed by activated macrophages to generate nitric oxide (NO) in a reaction mediated by nitric oxide synthase (NOSII) (Pereira et al., 1999; Munder et al., 1998). Finally trypanosomal ADC may afford protection against apoptotic death. Unfortunately no evidence of the presence of genes encoding these enzymes in the *T. cruzi* genome has yet been reported (Carrillo et al., 2003).

As mentioned above, L-methionine is the second amino acid precursor in polyamine metabolism. Methionine must be activated to *S*-adenosylmethionine (AdoMet) to take part in this pathway (Pérez-Pertejo et al., 2003). AdoMet is an intermediary metabolite involved in many *trans*-methylation reactions, and also participates in the *trans*-sulfuration pathway to cysteine, one of the three amino acids included in trypanothione, a glutathione-like antioxidant molecule in trypanosomatids. The role played by AdoMet in these three important pathways is related to the presence of the high energy sulfonium ion, which activates each of the attached carbons via nucleophilic attack. In *trans*-methylation reactions, the methyl group on the AdoMet molecule is yielded to a wide variety of acceptor substrates including DNA, phospholipids and proteins. *S*-Adenosylhomocysteine (AdoHcy) is the major by-product of these reactions. AdoHcy is a powerful competitive inhibitor of *trans*-methylation reactions; any decline in the AdoMet/AdoHcy ratio, whether as a result of an increase in AdoHcy levels or a decrease in AdoMet levels, is known to inhibit *trans*-methylation reactions. For this reason, cells try to maintain low levels of AdoHcy, for which purpose the removal of AdoHcy is essential. In *trans*-sulfuration reactions, the sulfur atom in the AdoMet molecule is converted—via a series of consecutive enzymatic reactions—to cysteine, a proteinogenetic amino acid, and glutathione. In Trypanosomatids (but not in *Plasmodium*) most of the glutathione is conjugated with spermidine to form the kinetoplastid-specific antioxidant trypanothione (N^1,N^8-bis(glutathionyl)-

spermidine), which accounts for about 70% of total glutathione. Trypanothione and trypanothione-dependent enzymes replace glutathione and glutathione-dependent enzymes present in a variety of prokaryotic and eukaryotic cells. The trypanothione system plays a vital role in Trypanosomatid cellular metabolism, in particular with respect to the intracellular redox balance and protection from the oxidative damage caused by free radicals and peroxides (for a recent review see: Muller et al. (2003).

AdoMet synthesis is a promising target for the treatment of disease caused by parasitic protozoa (Reguera et al., 2002). Several authors have sustained that AdoMet is an important metabolite in Trypanosomatids on the grounds of results obtained with the AdoMet-resembling antibiotic sinefungin (Neal et al., 1989; Phelouzat et al., 1993). Furthermore, long-term exposure of African trypanosomes to DFMO leads to a massive intracellular build-up of AdoMet and potential hypermethylation (Byers et al., 1991), causing cell death in the parasite, although this mechanism does not appear to be present in other parasites (Pérez-Pertejo et al., 2004).

AdoMet must be decarboxylated prior to involvement in polyamine metabolism. Putrescine-activated SAMDC (EC 4.1.1.50), a heterotetrameric self-cleaved enzyme with a pyruvoil group on its active site, has been cloned in *Leishmania* spp. (Roberts et al., 2002a) and *T. cruzi* (Kinch et al., 1999; Persson et al., 1998). Contrary to the experience with African and American trypanosomes, all attempts to measure leishmanial SAMDC have failed, raising doubts about its function in these microorganisms (Clyne et al., 2002; Tekwani et al., 1992). Double targeted gene replacement of the SAMDC gene recently revealed that this protein is absolutely requisite to cell growth in the absence of spermidine in *L. donovani* (Roberts et al., 2002a), ruling out any hypothetical auxotrophy for spermidine in these parasites. However, this enzyme seems to be absent in *Trichomonas vaginalis,* the agent causing human trichomoniasis, which is truly auxotrophic for spermidine (North et al., 1986).

The ODC and SAMDC activities in the malarial parasite *Plasmodium falciparum* have long been successfully assayed. Nonetheless, the genes encoding for the two activities were not identified until the RD Walter group at the Bernard-Koch Institute (Hamburg) reported the surprising finding that a single protein bears both ODC and SAMDC activities in a heterotetrameric form (Muller et al., 2000; Krause et al., 2000). This molecule coelutes with and forms a part of a 330 kDa protein that differs from the host counterpart in its response to putrescine. The N-terminal region of the newly synthesized protein contains a SAMDC proenzyme, which self-cleaves to produce an active enzyme with a catalytically essential pyruvoil residue at the new N-terminus. Since site-directed mutagenesis shows no domain–domain interactions between the two enzymes of the bifunctional *Plasmodium* ODC/SAMDC protein, it may be concluded that the two enzyme activities are independent of one another (Wrenger et al., 2001).

The condensation of one aminopropyl group to putrescine by spermidine synthase (Roberts et al., 2001) is the last enzyme-mediated step in polyamine biosynthesis by trypanosomatids. This enzyme has not been identified in *Trichomonas* (Yarlett and Bacchi, 1988), reinforcing the hypothesis of auxotrophy for spermidine. Unlike spermine synthase in mammalian hosts, the enzyme that transfers a second aminopropyl group to spermidine has not been identified in trypanosomatidae. Stage-dependent spermine pools have nonetheless been described in connection with intraerythrocytic growth in *Plasmodium* (Singh et al., 1997).

Although biochemical research relating to polyamine synthesis in parasites still remains open, few alternative routes or interconversion pathways have been described. Roberts et al., (2002b) cloned an agmatinase (agmatine ureohydrolase) gene from *L. mexicana*, which might explain why this amine is able to rescue arginase-null mutants (Reguera et al., personal communication), although the metabolic relevance is unclear because ADC (the only source of agmatine) is absent in the Leishmania genome. The polyamine metabolism of the urogenital parasite *T. vaginalis*, in turn, differs significantly from other eukaryotes in several aspects. Spermine from the mammalian cell is taken up by the parasite and back-converted to spermidine, via a spermidine/spermine N^1-acetyltransferase (SSAT)/ polyamine oxidase (PAO) compartmentalized coupled pathway. Cytosolic SSAT catalyses the transfer of an acetyl group from acetyl-CoA to a terminal aminopropyl nitrogen of spermine or spermidine to form N^1-acetyl spermine or N^1-acetyl spermidine, respectively. Finally, these are metabolized to spermidine and putrescine by hydrogenosomal polyamine oxidases (Yarlett et al., 2000).

3. Polyamine transport systems

Polyamines are protonated molecules carrying positive charges on each nitrogen atom at physiological or cellular pH. Many authors have compared these molecules to Ca^{2+} or Mg^{2+}, but they differ from such cations in the homogeneous distribution of the charge along the carbon chain (Seiler et al., 1996; Wallace et al., 2003). This rules out the possibility of passive sequestration by any cellular system. For that reason many researchers in the last decade have focused their efforts on the quest for polyamine carriers. Despite these efforts, polyamine transporters have only been cloned in bacteria and yeast.

3.1. Polyamine carriers in bacteria and yeast

Bacteria use two systems for polyamine uptake, a putrescine-specific system and a spermidine-preferential system (revised by Igarashi and Kashigawi, 1999, 2001). Both are ABC (ATP Binding Cassette) transporters, consisting of a substrate-binding protein in the periplasm, two channel-forming proteins (porins) and an inner-plasma

membrane side protein with ATPase activity to supply energy (Fig. 2A). The operon for the putrescine-specific transporter is mapped at 19 min on the *Escherichia coli* chromosome and comprises four genes encoding, respectively, for a substrate-binding protein (PotF), two porins (PotH and PotI) and an ATPase (PotG). The spermidine-preferential system, in turn, mapped at 26 min on the *E. coli* chromosome, contains a spermidine-binding protein (PotD), two porins (PotB and PotC) and the ATPase-encoding gene (PotA). The PotD in the spermidine-preferential system can bind both spermidine and putrescine, although its affinity for the latter is ten-fold lower. By contrast, the PotF in the putrescine-specific system does not recognize spermidine at all.

A third polyamine transport system has been described in some species of bacteria. The PotE protein encoded by the *potE* gene, together with *speF* (a gene encoding inducible ODC), constitute an operon in *E. coli* (Kashiwagi et al., 2000). The PotE transporter catalyzes the influx and efflux of putrescine and, as in other similar systems in bacteria (lysine/cadaverine, arginine/agmatine), can work as an antiporter system to exchange putrescine for its amino acid precursor ornithine.

In yeast a Transporter for Polyamines 1 (*TPO1*) gene encodes for a transmembrane protein, reminiscent in some ways of the PotE protein in bacteria (Tomitori et al., 2001; McNemar et al., 2001). The protein encoded by this gene has a long serine- and threonine-rich N-terminus domain, indicative of a post-translational regulatory mechanism mediated by serine or threonine kinases (Kakinuma et al., 1995). Four putative polyamine transporters, Tpo1p–Tpo4p, are found in the vacuolar membrane system to compartmentalize spermidine and spermine into vacuoles, inasmuch as free cytosolic polyamines are toxic for yeast (Tomitori et al., 1999). The carriers encoded by *TPO2* and *TPO3* are specific for spermine, whereas the transporters encoded by *TPO1* and *TPO4* recognize spermidine and spermine both: all are members of a family of multidrug-resistant carriers. Characterization studies of a *TPO1* deletion mutant have shown that Tpo1p is a spermidine exporter when cells are stressed with high spermidine concentrations (Albertsen et al., 2003).

3.2. Polyamine transport in mammalian cells

Polyamine transport mechanisms in mammals, unlike their counterparts in yeast and bacteria, are poorly characterized at the molecular level. Polyamine transport, uptake and efflux are net energy-consuming and saturable processes that can transport polyamines against significant concentration gradients, which suggests the existence of carrier-mediated mechanisms (see reviews: Seiler and Dezeure (1990); Khan et al. (1991); Grillo and Colombatto (1994); Seiler et al. (1996)).

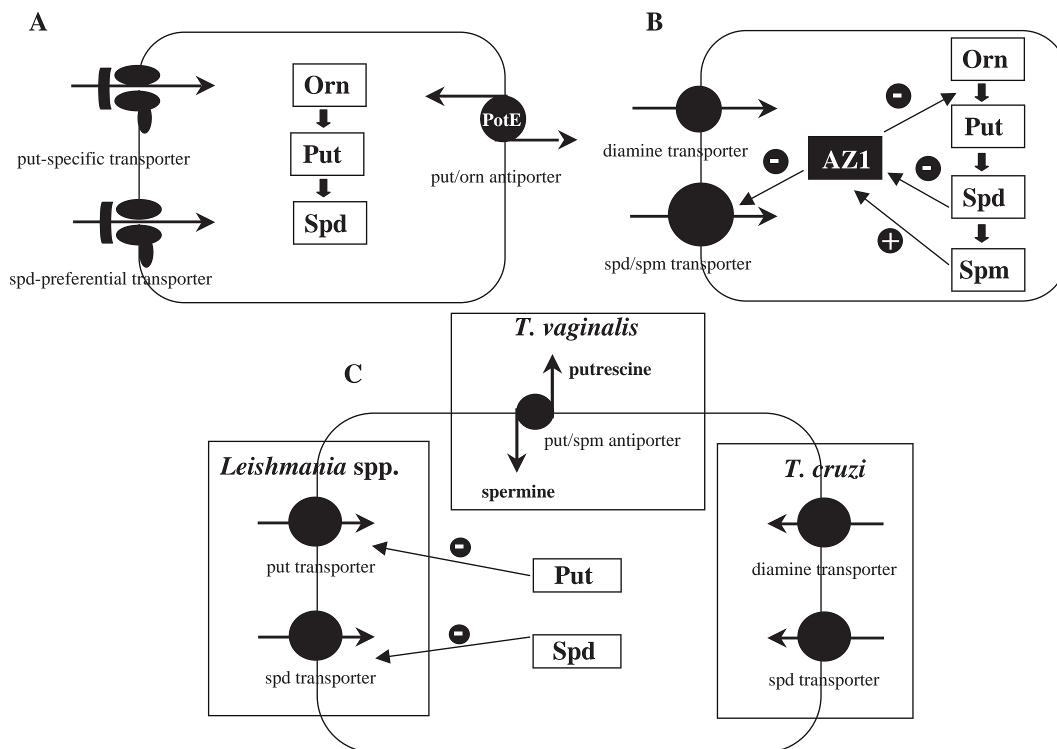

Fig. 2. Schematic representation of polyamine uptake systems in different organisms. (A) Different ABC-polyamine carriers in bacteria (according to Igarashi and Kashigawi, 1999) (B) Polyamine uptake is regulated with ODC by means an antizyme protein AZ in mammals. (C) Different polyamine uptake systems in parasites. *Trypanosoma cruzi* has an efficient diamine transport system for rescuing polyamine auxothrophy. On the other hand African trypanosomes lacks of an efficient active carrier for polyamines.

Separate transporters for putrescine on the one hand and spermidine and spermine on the other have been identified in mammalian cells. Uptake and excretion are catalyzed by one and the same polyamine transporter (Sakata et al., 2000). A similar reversal of roles has also been observed in *E. coli* PotE, which catalyses both the uptake and excretion of putrescine (Kashiwagi et al., 1997). In animal cells, excess polyamines are metabolized to acetyl derivatives, which are better substrates for excretion than the polyamines themselves, although polyamines are better substrates for uptake than acetylpolyamines (Seiler, 1987).

Polyamine transport is fully integrated into the regulatory system controlling polyamines in mammalian cells. As a general rule, factors increasing polyamine formation enhance their uptake from the extracellular environment, and conversely, situations where there is an excessive build-up of intracellular polyamines favor their removal. Several proteins involved in polyamine transport regulation fine-tune polyamine levels in the cell. The ODC-antizyme (AZ) is a small polyamine-induced protein that plays a role in ODC inhibition by forming an AZ–ODC complex. This complex is rapidly degraded by the 26 S proteasome in an ubiquitin-independent system (Murakami et al., 1992). Furthermore, AZ has been found to negatively regulate polyamine transport. The C-terminal half of AZ (residues 121–227) is sufficient to bind ODC (Ichiba et al. 1994; Li and Coffino, 1994) and inhibit polyamine transport (Sakata et al., 1997). The AZ protein is fairly labile and the content of this molecule in the cell is subject to positive regulation by polyamines through a ribosomal frameshift mechanism (Ivanov et al., 2000), negative regulation by an antizyme inhibitor protein (an ODC-resembling protein which lacks ODC activity) and post-translational proteolytic modifications (Murakami et al., 1996).

The excretion of polyamines is likewise a carrier-mediated process. Two systems seem to be involved in the diamine/polyamine efflux: a diamine exporter (AZ-independent process) which might be involved in the excretion of putrescine and *N*-acetylpolyamines (Xie et al., 1997); and the AZ-regulated polyamine transporter involved in the excretion of spermidine and spermine.

Recent studies indicate that agmatine, a biogenic amine that links the polyamine biosynthesis pathway and the generation of nitric oxide, can induce AZ, with the concomitant suppression of polyamine biosynthesis and transport (Satriano et al., 2001). This mechanism does not seem to be universal, however, because in other cell lines agmatine induces SSAT and inhibits ODC activity and inward polyamine transport without AZ involvement (Vargiu et al., 1999).

4. Polyamine uptake in parasites

As mentioned above, polyamines are synthesized "de novo" by most parasitic eukaryotes (with the exception of *T. cruzi*). Synthesis rates seem to replenish intracellular polyamine pools for optimal growth rates, and to adapt cells to needs. However, although "de novo" synthesis is the major source of polyamines, transport in and out of the cell also contributes to polyamine homoeostasis (Wallace, 1996). Membrane transporters can draw these compounds from the extracellular surroundings, i.e. blood or parasitoforous vacuoles. These uptake systems have been described in protozoan parasites at the biochemical level but their molecular characterization and cellular regulation are far from being understood.

The first evidence of an influx system for polyamines in parasites was reported by Kaur et al. (1986), who showed the effect of exogenous putrescine on leishmanial cells exposed to DFMO. Balaña-Fouce et al. (1989) provided the first description of an uptake system for protozoan parasites in *L. infantum* promastigotes. These authors showed that this organism is able to take up putrescine by a saturable, concentration- and energy-dependent, specific carrier not shared by higher polyamines, i.e. spermidine and spermine, other analogous polyamine such as MGBG, or basic amino acids. These results were conclusively confirmed in *L. donovani*, the etiological agent of visceral leishmaniasis, in which a second polyamine carrier was described, in this case for spermidine (Kandpal and Tekwani, 1997). The influx of this latter polyamine was found to be over seven times more effective than the uptake of putrescine, a diamine found not to share this mechanism, unlike spermine, which was observed to significantly inhibit spermidine uptake. Spermine, in turn, was not taken up at all by the putrescine transport system, and only moderately by the spermidine carrier. These findings were also observed in *L. mexicana* promastigotes (González and Algranati, 1994) and *C. fasciculata* parasites (Calonge et al., 1996a). In all these cases, putrescine uptake was specific and only scantly inhibited by spermidine, spermine and a number of variable length diamines. A common feature in all the polyamine transporters described for parasites to date is their dependence on the protonmotive force to take up the substrate. The fact that sodium ionophores, uncoupling agents and sulfhydryl group reagents were found to significantly inhibit polyamine influx in all the parasites studied suggests that the mechanism involved is coupled to a sodium electrochemical gradient (Balaña-Fouce et al., 1989; Kandpal and Tekwani, 1997).

The amastigote forms of *Leishmania* spp. and *T. cruzi* are intracellular parasites that invade and replicate within host macrophages. Whilst leishmanial cells have all the polyamine biosynthetic machinery, *T cruzi* is a putrescine auxotroph and dependent therefore on the host polyamine content—in the macrophage parasitoforous vacuoles in the amastigote stage (González et al., 1992) and in the mosquito gut in the epimastigote stage (Hunter et al., 1994). Early reports indicated that *T. cruzi* epimastigotes expressed a diamine putrescine/cadaverine influx system 10–50 times more efficient than the mechanism found in other trypano-

somatids (i.e. *Leishmania* or *Crithidia*) (González et al., 1992). Putrescine and cadaverine were both observed to be taken up by these epimastigotes, although 20- to 50-fold more cadaverine than putrescine was found to accumulate (Bacchi et al., 2001). Le Quesne and Fairlamb (1996) described at least two diamine/polyamine transporters with different affinities for putrescine and spermidine. Cell death produced by free radicals accumulation is the main consequence of trypanothione depletion induced by polyamine auxotrophy. Free glutathione, glutathionylspermidine and trypanothione are readily recovered in polyamine-supplemented cultures of *T. cruzi* epimastigotes after starvation (Ariyanayagam and Fairlamb, 1997).

It has long been known that after malaria infection the basic membrane transport properties of the parasitized erythrocyte are substantially altered. New permeation pathways appear in the infected cell whose properties are quite unlike those of any of the endogenous red cell systems (see review by Kirk (2001)). The "de novo" polyamine biosynthetic pathway is lacking in normal erythrocytes and only traces of spermidine and spermine are detectable (Assaraf et al., 1984). The dependence of polyamine uptake in red blood cells on the extracellular polyamine content is indicative of a diffusive process (Moulinoux et al., 1984). A carrier-mediated diffusive mixed process driven by a favorable concentration gradient has been reported for putrescine and spermidine in human erythrocytes (Fukumoto and Byus, 1996), however. The intra-erythrocytic schizogonous forms (trophozoites, ring forms and schizonts) of *P. knowlesi*-infected erythrocytes (the etiological agent of monkey's malaria) overexpress the putrescine transport system. The influx of the polyamine putrescine, although not spermidine or spermine, increases in parasitized erythrocytes via a saturable pathway in which the K_m is similar to the value for the putrescine transporter in normal erythrocytes but with a $V_{\max}$ some threefold higher than in uninfected cells (Singh et al., 1997). Some authors have suggested that choline and putrescine share the same carrier (both are cations at physiological pH) and that the rise in the transport rate of the two substrates may be attributed to the increased activity of a single class of carrier (Kirk, 2001).

Unlike other trypanosomatids, *Trichomonas* lacks SAMDC activity, although ODC has been successfully assayed, and putrescine levels are high, thus proving that this parasite is auxotrophic for spermidine. *T. vaginalis* parasitizes an area rich in polyamines, producing and excreting large amounts of putrescine via an energy-generating arginine dihydrolase pathway (Yarlett et al., 2000). Where large amounts of spermine are present, putrescine must be readily effluxed to the vaginal fluid. This parasite has developed a putrescine/spermine antiporter mechanism resembling the PotE system described in bacteria, which selectively transports 1 mol spermine into the cell while exporting 2 mol putrescine, effectively balancing the counterion charge (Yarlett and Bacchi, 1994). This spermine serves as a substrate for SSAT and polyamine oxidase, respectively forming N^1-acetylspermine and spermidine (Yarlett et al., 2000).

The molecular characterization of polyamine transporters in protozoa is a field open to research. No gene with this putative function has been included in any of the major genome databases to date. However, the B. Ullman group (Oregon Health and Science University, USA) has reported the existence of a gene in *L. major* encoding a polyamine transporter (*LmPOT1*). *LmPOT1* is a single gene located on chromosome 14 with 11 transmembrane domains. When functionally expressed in *Xenopus laevis* oocytes, the protein showed a high affinity for putrescine and spermidine. Moreover, pentamidine (an antiparasitic drug) was able to block this process (see below) (Hasne and Ullman, 2005).

5. Regulation of polyamine uptake

The spermidine-preferential uptake operon is transcriptionally regulated in bacteria by the PotD protein, synthesized as a precursor that down-regulates the expression of polyamine transporters (Antognoni et al., 1999). Eukaryotic cells seem to regulate polyamine uptake at the post-transcriptional level. Yeast Tpo1 transporters contain a long, serine- and threonine-rich hydrophilic N-terminal extension suitable for up-regulation by serine/theronine protein kinases (Kakinuma et al., 1995). Mammalian cells can down-regulate polyamine transport by AZ, which not only arrests polyamine uptake but also stimulates excretion of these metabolites (Sakata et al., 1997).

None of these mechanisms have yet been described in protozoa (Hua et al., 1995). However, insofar as genomic material is only expressed at the post-transcriptional level, the mechanisms involved are more likely to be similar to yeast or mammalian rather than prokaryotic systems (Teixeira and DaRocha, 2003).

A distinction should be drawn between developmental regulation (including stage in the life cycle and proliferative status) and metabolic adaptation to environmental changes. Digenetic protozoa, such as *Leishmania* and *T. cruzi*, can adapt the polyamine uptake to the environmental circumstances prevailing in their different life stages. Basselin et al. (2000) showed that promastigotes and amastigotes express different polyamine uptake systems depending on the extracellular pH. The amastigote transporter operates optimally at pH 5.5, whereas the promastigote transporter is more effective at pH 7.4, thus supporting the hypothesis of different polyamine transporters at different stages in the parasite's life cycle. *T. cruzi* must adapt its diamine uptake when it invades host cells with a polyamine concentration significantly higher than in the serum. Overall, epimastigotes, trypomastigotes and amastigotes are similar in their abilities to assimilate diamines and polyamines, with higher build-ups of

spermidine and cadaverine than putrescine (Bacchi et al., 2001). Surprisingly these authors found a low but detectable stage-dependent influx of spermine during the epimastigote and amastigote stages of the life cycle.

Both *L. infantum* and *C. fasciculata* modulate the expression of their transporters in keeping with their proliferative status (Balaña-Fouce et al., 1991b; Calonge et al., 1996b). In synchronized cultures of both species, polyamine transport peaked in the early log phase, to drop sharply to nearly undetectable values during the stationary phase. Such changes were also found by Le Quesne and Fairlamb (1996) in *T. cruzi* epimastigotes, where a substantial 10-fold rise in putrescine transport V_{max} in the mid-exponential phase was observed along with no significant changes in the affinity constant, suggesting "de novo" carrier synthesis. Furthermore, this change in the uptake rate of putrescine was due to a highly inducible protein transporter, whose half-life in the presence of protein synthesis inhibitors (i.e., actinomycin D and cycloheximide) was estimated to be 18 h, considerably higher than the value calculated by Kaouass et al. (1998) for yeast (10–15 min).

Both ODC activity and polyamine uptake appear to be controlled by the intracellular concentration of polyamines. The depletion of intracellular polyamines by DFMO enhances uptake rates. Conversely, ODC-overexpressing cells are able to down-regulate polyamine influx to non-toxic rates. The same scenario can be found in eukaryotic parasites. Drug-depletion of intracellular putrescine pools or an excess of polyamines in the medium can regulate carrier expression. Administering DFMO to *L. infantum* promastigotes produces a steep and effective decline in intracellular putrescine pools, originating a rapid rise of putrescine uptake, estimated to increase by over ten-fold (Balaña-Fouce et al., 1991b). Since such induction modifies the V_{max} of the transporter but not the K_m and it can be inhibited by cycloheximide, the process would appear to involve new carrier synthesis. Furthermore, the exogenous addition of putrescine leads to down-regulation of the inward transport of this diamine, a development related to the replenishment of intracellular putrescine and spermidine pools (Calonge et al., 1996a).

The up-regulation of polyamine transport is of maximum relevance to the polyamine-based therapy of parasitic disease. DFMO is cytostatic rather than cytotoxic and the death of invading parasites mediated by this drug is not due solely to ODC inhibition, but to other biochemical and immunological processes (Fairlamb et al., 1987). In fact, the cytostatic effect caused by DFMO can be circumvented by exogenous putrescine, making this the step where the induction of polyamine transport plays an important role in chemotherapy. Up-regulation of polyamine import would explain the effectiveness of DFMO against refractory SNC sleeping sickness caused by bloodstream trypanosomatids viz *T. brucei gambiense* or *T. brucei brucei*, and the scant success of this compound in combating intracellular parasites such as *Leishmania* spp. or *Plasmodium* spp. African trypanosomes treated with DFMO need exogenous putrescine or spermidine to grow. Host blood is a poor source of polyamines (Loser et al., 1990) and the polyamine transporter is scarce and non-inducible in these parasites (Bacchi and Yarlett, 1993; González et al., 1992).

T. cruzi and *Trichomonas* parasites are of particular interest in this regard. Putrescine transport is not up-regulated by DFMO in these microorganisms as it is in other protozoa. Since *T. cruzi* lacks ODC it is not vulnerable to putrescine depletion by DFMO (González et al., 1992) and *Trichomonas*, in turn, has a spermine/putrescine antiporter which is neither up- nor down-regulated. No changes are observed in antiporter expression in DFMO-treated *Trichomonas* cultures (Yarlett and Bacchi, 1994).

Polyamine requirements for the normal proliferation of trypanosomatids can be met by spermidine alone, probably due to its unique role as substrate for trypanothione synthesis (González et al., 2001). Double replacement techniques recently applied to generate null mutants in polyamine biosynthetic enzymes have shown the importance of polyamine uptake in rescuing putrescine and spermidine auxotrophy in genetically-modified parasites. The Δodc-knockout strain of *L. donovani*, lacking both ODC alleles, was auxotrophic for polyamines. The conditionally lethal Δodc mutant could survive if supplemented with either putrescine or spermidine, but not with spermine (Jiang et al., 1999). Similar results have been reported for *T. brucei*, where the permanent auxotrophy of null-mutants had to be remedied with polyamines (thus suggesting the presence of a polyamine transporter in this parasite). Null-ODC mutants grown in a spermidine-supplemented medium exhibited a steep reduction in intracellular putrescine; supporting the assertion that spermidine is necessary and sufficient to satisfy the parasite's polyamine requirements (Li et al., 1996). This ODC-deficient strain was unable to multiply in mice and was quickly eliminated from the blood because of the low levels of serum polyamines. Nonetheless, infected mice proved to be immuno-protected from new exposures to wild-type *T. brucei* of the same serotype (Mutomba et al.,1999).

Leishmania null-mutants in spermidine synthesis, Δadometdc (Roberts et al., 2002b) and Δspds (Roberts et al., 2001) mutants were likewise unable to grow in polyamine-deficient media. The resulting parasites were auxotrophic for spermidine, a requirement that could only be filled by adding exogenous spermidine to the culture medium; putrescine, spermine and other polyamines were ineffective in this respect.

Roberts et al. (2004) recently generated an arginase null-mutant of *L. mexicana*, Δarg; arginase is the enzyme that mediates in the transformation of arginine to ornithine and subsequently to polyamines in trypanosomatids. ΔArg is auxotrophic for ornithine as well as for putrescine and spermidine. And yet no differences were found in the virulence of the mutant and wild-type strains, thus showing

the importance of extracellular polyamines for rescuing the original infectiveness of these parasites (Reguera et al. personal communication).

6. Inhibition of polyamine uptake

The earliest studies of polyamine uptake inhibition in parasites were conducted on aromatic diamidines (pentamidine, berenil, CGP40215 and others) (Balaña-Fouce et al., 1989; Reguera et al., 1994; Calonge et al., 1996b; Mukhopadhyay et al., 1996). These compounds are considered to be pleiotropic, not only inhibiting polyamine and arginine uptake non-competitively (Kandpal et al., 1995, 1996), but displacing spermidine from nucleic acids (Johnson et al., 1998) and inhibiting SAMDC (Balaña-Fouce et al., 1986; Bitonti et al., 1986; Mukhopadhyay and Madhubala, 1995a). QSAR studies using a series of cationic diamidines showed that the inhibition of polyamine uptake was proportional to the distance between the amino moieties in the amidino substituents (Navas et al., 1996). Some early studies suggested that the polyamine transport system drives the influx of pentamidine into *Leishmania* promastigotes. Damper and Patton (1976) described pentamidine uptake as a saturable, carrier-mediated, high-affinity, energy-dependent process which was inhibited by exogenous polyamines in *L. donovani* and *L. amazonensis* promastigotes and axenic amastigotes. Moreover, polyamine metabolism and uptake were seen to be altered in pentamidine-resistant promastigotes, whose permeability to putrescine and spermidine was lower than in wild-type promastigotes. However, further studies ruled out this possibility. In fact, more than 50% of the pentamidine content of bloodstream *T. brucei* was found to be transported by the P2 adenosine/adenine transporter, whilst the remaining drug was taken up by two unidentified carriers (de Koning, 2001). Procyclic forms of these parasites can express a fourth specific transporter not shared by polyamines. Along these same lines, Basselin et al. (2000) working with *L. amazonensis* promastigotes and axenic amastigotes, suggested that although pentamidine inhibits polyamine uptake non-competitively, since putrescine and spermidine have no effect on pentamidine influx, polyamine transporters must not be the mechanism for carrying pentamidine into the cells (Basselin et al., 2002), a finding similar to what has been observed in connection with berenil (Balaña-Fouce et al., 1991a). Unlike *T. brucei*, Leishmania spp. have no functional equivalent to the P2 adenosine/adenine transporter, from which it may be deduced that the mechanisms involved differ from those described in trypanosomes (Landfear, 2001).

Polyamine analogues are powerful competitive inhibitors of polyamine influx. Bis(benzyl)polyamine analogues were found to inhibit both the putrescine and spermidine/spermine uptake systems in filarial worms (Muller et al., 1991). When Mukhopadhyay and Madhubala (1993) assayed these compounds in experimental *L. donovani* infections, they were found to inhibit ODC and SAMDC activities and deplete the intracellular levels of polyamines (Mukhopadhyay and Madhubala, 1995b). They were also successfully tested “in vitro” against *P. falciparum* alone, and in combination with DFMO and chloroquine (Bitonti et al., 1989; Das et al., 1995), against *T. cruzi* (Majumder and Kierszenbaum, 1993), and antimony-susceptible and resistant strains of *L. donovani* (Baumann et al., 1990, 1991).

Other polyamine analogues that alter polyamine uptake in cancer cells and should be tested with parasites are the bis(ethyl)-derivatives of spermidine and spermine viz. N^1,N^{12}-bis(ethyl)spermine, N^1,N^{11}-bis(ethyl)norspermine, and N^1,N^{14}-bis(ethyl)homospermine. Several cancer cell studies showed that these compounds strongly suppress polyamines by down-regulating polyamine biosynthetic enzymes as a result of the massive induction of SSAT (Parry et al., 1995) and the increase in the excretion of spermidine and N^1-acetylspermidine to the medium (Pegg et al., 1989). Unlike tumor cells, *Trichomonas* can be targeted by these compounds but SSAT activity is significantly inhibited, which is indicative of differences in the pathways followed in parasite and host cells (Yarlett et al., 2000). After successful testing in recent trials against *T. cruzi* epimastigotes, a series of these compounds have a promising future as therapeutic drugs (Zou et al., 2001).

Some spermine/amino acid conjugates can inhibit the uptake of spermidine by MDA-MB-231 breast cancer cells. In combination with DFMO, they induce cytostatic growth arrest in a variety of cancer cells, even when used in the presence of an extracellular source of transportable spermidine (Weeks et al., 2000; Burns et al., 2001).

N-acetyl derivatives of spermidine N^1-acetylspermidine and N^8-acetylspermidine also competitively inhibit the uptake of spermine, the latter more effectively. Moreover, the above analogues inhibit one of the putrescine uptake carriers, also competitively, suggesting the existence of a common transporter for both putrescine and spermine.

7. Dietary polyamine deprivation

Polyamine blood levels are of great relevance in the development of proliferative processes. Serum polyamines may be the outcome of internal metabolism or, more frequently, come from external sources such as dietary habits or gut microflora. There is no evidence of polyamine bioavailability from these external sources in healthy animals, although they seem to be a major contributor to the total body burden (Bardocz et al., 1995).

Polyamines contained in food or formed by intestinal microflora are absorbed by specific carriers sited in the mucosa of the small intestinal or colon and constitute the most important source of blood polyamines. In the mid 1990s a new strategy was postulated, consisting in the systematic blockade of all endogenous and exogenous sources of polyamines “in vivo”. It was proposed that

administering a cocktail of polyamine biosynthesis inhibitors, along with non-absorbable antibiotics (neomycin and metronidazole) to decontaminate the gastrointestinal tract, to animals fed a low polyamine diet would inhibit proliferative processes. Some promising results have been reported in experimental and clinical trials. Treatment with a cocktail of polyamine inhibitors and a polyamine-deficient diet prevented tumor-induced immune suppression (Chamaillard et al., 1997) in mice grafted with the 3LL (Lewis lung) carcinoma, as well as pre-neoplasic changes in the intestine of chemically-induced cancer of the colon in rats (Duranton et al., 1997).

Nishimura et al. (2001) assayed this novel approach in an experimental model for treating African trypanosomiasis. Dietary polyamines were reduced by feeding rats a polyamine-deficient chow (PDC). The serum polyamine content dropped in animals fed with PDC and with it the proliferation of bloodstream trypanosomes. PDC arrested trypanosome propagation and the symptoms of infection—weight loss and anemia—improved considerably. Survival was extended in these rats. These results show that polyamine biosynthesis is insufficient to fully meet the needs of parasites growing "in vivo", which seem to require some outside source of these metabolites.

8. Concluding remarks

It was more than 8 years ago than the last review on polyamine uptake was reported by Seiler et al. (1996) where the authors claimed to the scientific community in this field for the molecular characterization of polyamine transporters in eukaryotic cells as the only way to determine the actual role played by this system in cellular physiology. Much effort has been made during this period in improving molecular biology techniques, but despite this no genetic sequence encoding a polyamine carrier in parasites has been deposited in any major database. New genomic/proteomic approaches can help to elucidate the physiological functions of the genes/proteins induced in polyamine auxothrophic cell lines, created by double-replacement techniques or small interference RNA, which can act as polyamine transporters in parasites, and to determine if they are essential. Further, heterologous expression of cloned cDNAs in Xenopus eggs will permit the functional expression of those promising genes previously identified as polyamine carriers and should establish the basis of their future molecular characterization in parasites.

The integral regulation of polyamine metabolism and uptake of each parasitic protozoa and its role played in the host/parasite relationship will be crucial for the use and design of polyamine-based drugs. To this goal will contribute future studies on the three-dimensional structures of the polyamine carriers, which will encourage QSAR studies based on the interaction of the inhibitor with the transporter protein.

Acknowledgments

This research was supported in part by Comisión Interministerial de Ciencia y Tecnología (grants BMC2002-04107-C02-02 and AGL2003-06976) and Junta de Castilla y León (grants Le54/03 and Le04/03).

References

Albertsen, M., Bellahn, I., Kramer, R., Waffenschmidt, S., 2003. Localization and function of the yeast multidrug transporter Tpo1p. J. Biol. Chem. 278, 12820–12825.

Antognoni, F., Del Duca, S., Kuraishi, A., Kawabe, E., Fukuchi-Shimogori, T., Kashiwagi, K., Igarashi, K., 1999. Transcriptional inhibition of the operon for the spermidine uptake system by the substrate-binding protein PotD. J. Biol. Chem. 274, 1942–1948.

Ariyanayagam, M.R., Fairlamb, A.H., 1997. Diamine auxotrophy may be a universal feature of *Trypanosoma cruzi* epimastigotes. Mol. Biochem. Parasitol. 84, 111–121.

Assaraf, Y.G., Golenser, J., Spira, D.T., Bachrach, U., 1984. Polyamine levels and the activity of their biosynthetic enzymes in human erythrocytes infected with the malarial parasite, *Plasmodium falciparum*. Biochem. J. 222, 815–819.

Bacchi, C.J., Yarlett, N., 1993. Effects of antagonists of polyamine metabolism on African trypanosomes. Acta Trop. 54, 225–236.

Bacchi, C.J., Yarlett, N., 2002. Polyamine metabolism as chemotherapeutic target in protozoan parasites. Mini Rev. Med. Chem. 2, 553–563.

Bacchi, C.J., Nathan, H.C., Hutner, S.H., McCann, P.P., Sjoerdsma, A., 1980. Polyamine metabolism: a potential target in trypanosomes. Science 210, 332–334.

Bacchi, C.J., Braunstein, V.L., Rattendi, D., Yarlett, N., Wittner, M., Tanowitz, H.B., 2001. Stage-specific polyamine metabolism in *Trypanosoma cruzi*. J. Eukaryot. Microbiol. Suppl., 201S–202S.

Balaña-Fouce, R., Garzon Pulido, T., Ordoñez-Escudero, D., Garrido-Pertierra, A., 1986. Inhibition of diamine oxidase and *S*-adenosylmethionine decarboxylase by diminacene aceturate (Berenil). Biochem. Pharmacol. 35, 1597–1600.

Balaña-Fouce, R., Ordoñez, D., Alunda, J.M., 1989. Putrescine transport system in *Leishmania infantum* promastigotes. Mol. Biochem. Parasitol. 35, 43–50.

Balaña-Fouce, R., Escribano, M.I., Alunda, J.M., 1991a. Putrescine uptake regulation in response to alpha-difluoromethylornithine treatment in *Leishmania infantum* promastigotes. Mol. Cell. Biochem. 107, 127–133.

Balaña-Fouce, R., Escribano, M.I., Alunda, J.M., 1991b. *Leishmania infantum*: polyamine biosynthesis and levels during the growth of promastigotes. Int. J. Biochem. 23, 1213–1217.

Bardocz, S., Duguid, T.J., Brown, D.S., Grant, G., Pusztai, A., White, A., Ralph, A., 1995. The importance of dietary polyamines in cell regeneration and growth. Br. J. Nutr. 73, 819–828.

Basselin, M., Coombs, G.H., Barrett, M.P., 2000. Putrescine and spermidine transport in *Leishmania*. Mol. Biochem. Parasitol. 109, 37–46.

Basselin, M., Denise, H., Coombs, G.H., Barrett, M.P., 2002. Resistance to pentamidine in *Leishmania mexicana* involves exclusion of the drug from the mitochondrion. Antimicrob. Agents Chemother. 46, 3731–3738.

Baumann, R.J., Hanson, W.L., McCann, P.P., Sjoerdsma, A., Bitonti, A.J., 1990. Suppression of both antimony-susceptible and antimony-resistant *Leishmania donovani* by a bis(benzyl)polyamine analog. Antimicrob. Agents Chemother. 34, 722–727.

Baumann, R.J., McCann, P.P., Bitonti, A.J., 1991. Suppression of *Leishmania donovani* by oral administration of a bis(benzyl)polyamine analog. Antimicrob. Agents Chemother. 35, 1403–1407.

Bitonti, A.J., Dumont, J.A., McCann, P.P., 1986. Characterization of *Trypanosoma brucei brucei S*-adenosyl-L-methionine decarboxylase and its inhibition by Berenil, pentamidine and methylglyoxal bis(guanylhydrazone). Biochem. J. 237, 685–689.

Bitonti, A.J., Dumont, J.A., Bush, T.L., Edwards, M.L., Stemerick, D.M., McCann, P.P., Sjoerdsma, A., 1989. Bis(benzyl)polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (*Plasmodium falciparum*) in vitro and in combination with alpha-difluoromethylornithine cure murine malaria. Proc. Natl. Acad. Sci. U. S. A. 86, 651–655.

Burns, M.R., Carlson, C.L., Vanderwerf, S.M., Ziemer, J.R., Weeks, R.S., Cai, F., Webb, H.K., Graminski, G.F., 2001. Amino acid/spermine conjugates: polyamine amides as potent spermidine uptake inhibitors. J. Med. Chem. 44, 3632–3644.

Burri, C., Brun, R., 2003. Eflornithine for the treatment of human African trypanosomiasis. Parasitol. Res. 90, S49–S52.

Byers, T.L., Bush, T.L., McCann, P.P., Bitonti, A.J., 1991. Antitrypanosomal effects of polyamine biosynthesis inhibitors correlate with increases in *Trypanosoma brucei brucei S*-adenosyl-L-methionine. Biochem. J. 274, 527–533.

Calonge, M., Cubría, J.C., Balaña-Fouce, R., Ordoñez, D., 1996a. Putrescine active uptake system in the Trypanosomatid *Crithidia fasciculata*. Biol. Chem. Hoppe-Seyler 377, 233–238.

Calonge, M., Johnson, R., Balaña-Fouce, R., Ordoñez, D., 1996b. Effects of cationic diamidines on polyamine content and uptake on *Leishmania infantum* in in vitro cultures. Biochem. Pharmacol. 52, 835–841.

Carrillo, C., Cejas, S., González, N.S., Algranati, I.D., 1999. *Trypanosoma cruzi* epimastigotes lack ornithine decarboxylase but can express a foreign gene encoding this enzyme. FEBS Letts. 454, 192–196.

Carrillo, C., Cejas, S., Cortes, M., Ceriani, C., Huber, A., González, N.S., Algranati, I.D., 2000. Sensitivity of trypanosomatid protozoa to DFMO and metabolic turnover of ornithine decarboxylase. Biochem. Biophys. Res. Commun. 279, 663–668.

Carrillo, C., Cejas, S., Huber, A., Gonzalez, N.S., Algranati, I.D., 2003. Lack of arginine decarboxylase in *Trypanosoma cruzi* epimastigotes. J. Eukaryot. Microbiol. 50, 312–316.

Ceriani, C., Gonzalez, N.S., Algranati, I.D., 1992. Ornithine decarboxylase from *Crithidia fasciculata* is metabolically unstable and resistant to polyamine down-regulation. FEBS Lett. 301, 261–264.

Chamaillard, L., Catros-Quemener, V., Delcros, J.G., Bansard, J.Y., Havouis, R., Desury, D., Commeurec, A., Genetet, N., Moulinoux, J.P., 1997. Polyamine deprivation prevents the development of tumour-induced immune suppression. Br. J. Cancer 76, 365–370.

Clyne, T., Kinch, L.N., Phillips, M.A., 2002. Putrescine activation of *Trypanosoma cruzi S*-adenosylmethionine decarboxylase. Biochemistry 41, 13207–13216.

Damper, D., Patton, C.L., 1976. Pentamidine transport in *Trypanosoma brucei*: kinetics and specificity. Biochem. Pharmacol. 25, 271–276.

Das, B., Gupta, R., Madhubala, R., 1995. Combined action of inhibitors of polyamine biosynthetic pathway with a known antimalarial drug chloroquine on *Plasmodium falciparum*. Pharmacol. Res. 31, 189–193.

De Koning, H.P., 2001. Uptake of pentamidine in *Trypanosoma brucei brucei* is mediated by three distinct transporters: implications for cross-resistance with arsenicals. Mol. Pharmacol. 59, 586–592.

Duranton, B., Nsi-Emvo, E., Schleiffer, R., Gosse, F., Galluser, M., Raul, F., 1997. Suppression of preneoplastic changes in the intestine of rats fed low levels of polyamines. Cancer Res. 57, 573–575.

Fairlamb, A.H., 2002. Metabolic pathway analysis in trypanosomes and malaria parasites. Philos. Trans. R. Soc. Lond., B 357, 101–107.

Fairlamb, A.H., Henderson, G.B., Bacchi, C.J., Cerami, A., 1987. In vivo effects of difluoromethylornithine on trypanothione and polyamine levels in bloodstream forms of *Trypanosoma brucei*. Mol. Biochem. Parasitol. 24, 185–191.

Fukumoto, G.H., Byus, C.V., 1996. A kinetic characterization of putrescine and spermidine uptake and export in human erythrocytes. Biochim. Biophys. Acta 1282, 48–56.

Ghoda, L., Phillips, M.A., Bass, K.E., Wang, C.C., Coffino, P., 1990. Trypanosome ornithine decarboxylase is stable because it lacks sequences found in the carboxyl terminus of the mouse enzyme which target the latter for intracellular degradation. J. Biol. Chem. 265, 11823–11826.

González, N.S., Algranati, I.D., 1994. Regulation of putrescine uptake in *Leishmania mexicana* promastigotes. Cell. Mol. Biol. (Noisy-le-grand) 40, 907–914.

González, N.S., Ceriani, C., Algranati, I.D., 1992. Differential regulation of putrescine uptake in *Trypanosoma cruzi* and other trypanosomatids. Biochem. Biophys. Res. Commun. 188, 120–128.

González, N.S., Huber, A., Algranati, I.D., 2001. Spermidine is essential for normal proliferation of trypanosomatid protozoa. FEBS Lett. 508, 323–326.

Grillo, M.A., Colombatto, S., 1994. Polyamine transport in cells. Biochem. Soc. Trans. 22, 894–898.

Hanson, S., Adelman, J., Ullman, B., 1992. Amplification and molecular cloning of the ornithine decarboxylase gene of *Leishmania donovani*. J. Biol. Chem. 267, 2350–2359.

Hasne, M.P., Ullman, B., 2005. Identification and characterization of a polyamine permease from the protozoan parasite *Leishmania major*. J. Biol. Chem. (in press).

Heby, O., Roberts, S.C., Ullman, B., 2003. Polyamine biosynthetic enzymes as drug targets in parasitic protozoa. Biochem. Soc. Trans. 31, 415–419.

Hernández, S., Schwarcz de Tarlovsky, S., 1999. Arginine decarboxylase in *Trypanosoma cruzi*, characteristics and kinetic properties. Cell. Mol. Biol. (Noisy-le-grand) 45, 383–391.

Hua, S.B., Li, X., Coffino, P., Wang, C.C., 1995. Rat antizyme inhibits the activity but does not promote the degradation of mouse ornithine decarboxylase in *Trypanosoma brucei*. J. Biol. Chem. 270, 10264–10271.

Hunter, K.J., Le Quesne, S.A., Fairlamb, A.H., 1994. Identification and biosynthesis of *N*1,*N*9-bis(glutathionyl)aminopropylcadaverine (homotrypanothione) in *Trypanosoma cruzi*. Eur. J. Biochem. 226, 1019–1027.

Ichiba, T., Matsufuji, S., Miyazaki, Y., Murakami, Y., Tanaka, K., Ichihara, A., Hayashi, S., 1994. Functional regions of ornithine decarboxylase antizyme. Biochem. Biophys. Res. Commun. 200, 1721–1727.

Igarashi, K., Kashiwagi, K., 1999. Polyamine transport in bacteria and yeast. Biochem. J. 344, 633–642.

Igarashi, K., Kashiwagi, K., 2001. Polyamine uptake systems in *Escherichia coli*. Res. Microbiol. 152, 271–278.

Ivanov, I.P., Gesteland, R.F., Atkins, J.F., 2000. Antizyme expression: a subversion of triplet decoding, which is remarkably conserved by evolution, is a sensor for an autoregulatory circuit. Nucleic Acids Res. 28, 3185–3196.

Jiang, Y., Roberts, S.C., Jardim, A., Carter, N.S., Shih, S., Ariyanayagam, M., Fairlamb, A.H., Ullman, B., 1999. Ornithine decarboxylase gene deletion mutants of *Leishmania donovani*. J. Biol. Chem. 274, 3781–3788.

Johnson, R., Cubría, J.C., Reguera, R.M., Balaña-Fouce, R., Ordoñez, D., 1998. Interaction of cationic diamidines with *Leishmania infantum* DNA. Biol. Chem. 379, 925–930.

Kakinuma, Y., Maruyama, T., Nozaki, T., Wada, Y., Ohsumi, Y., Igarashi, K., 1995. Cloning of the gene encoding a putative serine/threonine protein kinase which enhances spermine uptake in *Saccharomyces cerevisiae* Biochem. Biophys. Res. Comm. 216, 985–992.

Kandpal, M., Tekwani, B.L., 1997. Polyamine transport systems of *Leishmania donovani* promastigotes. Life Sci. 60, 1793–1801.

Kandpal, M., Balaña-Fouce, R., Pal, A., Guru, P.Y., Tekwani, B.L., 1995. Kinetics and molecular characteristics of arginine transport by *Leishmania donovani* promastigotes. Mol. Biochem. Parasitol. 71, 193–201.

Kandpal, M., Tekwani, B.L., Chauhan, P.M., Bhaduri, A.P., 1996. Correlation between inhibition of growth and arginine transport of *Leishmania donovani* promastigotes in vitro by diamidines. Life Sci. 59, 75–80.

Kaouass, M., Gamache, I., Ramotar, D., Audette, M., Poulin, R., 1998. The spermidine transport system is regulated by ligand inactivation, endocytosis, and by the Npr1p Ser/Thr protein kinase in *Saccharomyces cerevisiae*. J. Biol. Chem. 273, 2109–2117.

Kashiwagi, K., Shibuya, S., Tomitori, H., Kuraishi, A., Igarashi, K., 1997. Excretion and uptake of putrescine by the PotE protein in *Escherichia coli*. J. Biol. Chem. 272, 6318–6323.

Kashiwagi, K., Kuraishi, A., Tomitori, H., Igarashi, A., Nishimura, K., Shirahata, A., Igarashi, K., 2000. Identification of the putrescine recognition site on polyamine transport protein PotE. J. Biol. Chem. 275, 36007–36012.

Kaur, K., Emmett, K., McCann, P.P., Sjoerdsma, A., Ullman, B., 1986. Effects of DL-alpha-difluoromethylornithine on *Leishmania donovani* promastigotes. J. Protozool. 33, 518–521.

Khan, N.A., Quemener, V., Moulinoux, J.P., 1991. Polyamine membrane transport regulation. Cell. Biol. Int. Rep. 15, 9–24.

Kinch, L.N., Scott, J.R., Ullman, B., Phillips, M.A., 1999. Cloning and kinetic characterization of the *Trypanosoma cruzi S*-adenosylmethionine decarboxylase. Mol. Biochem. Parasitol. 101, 1–11.

Kirk, K., 2001. Membrane transport in the malaria-infected erythrocyte. Physiol. Rev. 81, 495–537.

Krause, T., Luersen, K., Wrenger, C., Gilberger, T.W., Muller, S., Walter, R.D., 2000. The ornithine decarboxylase domain of the bifunctional ornithine decarboxylase/*S*-adenosylmethionine decarboxylase of *Plasmodium falciparum*: recombinant expression and catalytic properties of two different constructs. Biochem. J. 352, 287–292.

Landfear, S.M., 2001. Molecular genetics of nucleoside transporters in *Leishmania* and African trypanosomes. Biochem. Pharmacol. 62, 149–155.

Le Quesne, S.A., Fairlamb, A.H., 1996. Regulation of a high-affinity diamine transport system in *Trypanosoma cruzi* epimastigotes. Biochem. J. 316, 481–486.

Li, F., Hua, S.B., Wang, C.C., Gottesdiener, K.M., 1996. Procyclic *Trypanosoma brucei* cell lines deficient in ornithine decarboxylase activity. Mol. Biochem. Parasitol. 78, 227–236.

Li, X., Coffino, P., 1994. Distinct domains of antizyme required for binding and proteolysis of ornithine decarboxylase. Mol. Cell. Biol. 14, 87–92.

Loser, C., Folsch, U.R., Paprotny, C., Creutzfeldt, W., 1990. Polyamine concentrations in pancreatic tissue, serum, and urine of patients with pancreatic cancer. Pancreas 5, 119–127.

Majumder, S., Kierszenbaum, F., 1993. Inhibition of host cell invasion and intracellular replication of *Trypanosoma cruzi* by *N*,*N*'-bis(benzyl)-substituted polyamine analogs. Antimicrob. Agents Chemother. 37, 2235–2238.

Majumder, S., Wirth, J.J., Bitonti, A.J., McCann, P.P., Kierszenbaum, F., 1992. Biochemical evidence for the presence of arginine decarboxylase activity in *Trypanosoma cruzi*. J. Parasitol. 78, 371–374.

McNemar, M.D., Gorman, J.A., Buckley, H.R., 2001. Isolation of a gene encoding a putative polyamine transporter from *Candida albicans*, GPT1. Yeast 18, 555–561.

Moulinoux, J.P., Le Calve, M., Quemener, V., Quash, G., 1984. In vitro studies on the entry of polyamines into normal red blood cells. Biochimie 66, 385–393.

Mukhopadhyay, R., Madhubala, R., 1993. Effect of a bis(benzyl)-polyamine analogue, and DL-alpha-difluoromethylornithine on parasite suppression and cellular polyamine levels in golden hamster during *Leishmania donovani* infection. Pharmacol. Res. 28, 359–365.

Mukhopadhyay, R., Madhubala, R., 1995a. Antileishmanial activity of berenil and methylglyoxal bis (guanylhydrazone) and its correlation with *S*-adenosylmethionine decarboxylase and polyamines. Int. J. Biochem. Cell. Biol. 27, 55–59.

Mukhopadhyay, R., Madhubala, R., 1995b. Effects of bis(benzyl)polyamine analogs on *Leishmania donovani* promastigotes. Exp. Parasitol. 81, 39–46.

Mukhopadhyay, R., Kapoor, P., Madhubala, R., 1996. Antileishmanial effect of a potent *S*-adenosylmethionine decarboxylase inhibitor: CGP 40215A. Pharmacol. Res. 33, 67–70.

Muller, S., Luchow, A., McCann, P.P., Walter, R.D., 1991. Effect of bis(benzyl)polyamine derivatives on polyamine transport and survival of *Brugia pahangi*. Parasitol. Res. 77, 612–615.

Muller, S., Da'dara, A., Luersen, K., Wrenger, C., Das, B., Gupta, R., Madhubala, R., Walter, R.D., 2000. In the human malaria parasite *Plasmodium falciparum*, polyamines are synthesized by a bifunctional ornithine decarboxylase, *S*-adenosylmethionine decarboxylase. J. Biol. Chem. 275, 8097–8102.

Muller, S., Coombs, G.H., Walter, R.D., 2001. Targeting polyamines of parasitic protozoa in chemotherapy. Trends Parasitol. 17, 242–249.

Muller, S., Liebau, E., Walter, R.D., Krauth-Siegel, R.L., 2003. Thiol-based redox metabolism of protozoan parasites. Trends Parasitol. 19, 320–328.

Munder, M., Eichmann, K., Modolell, M., 1998. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD41 T cells correlates with Th1/Th2 phenotype. J. Immunol. 160, 5347–5354.

Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., Tanaka, K., Ichihara, A., 1992. Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. Nature 360, 597–599.

Murakami, Y., Ichiba, T., Matsufuji, S., Hayashi, S., 1996. Cloning of antizyme inhibitor, a highly homologous protein to ornithine decarboxylase. J. Biol. Chem. 271, 3340–3342.

Mutomba, M.C., Li, F., Gottesdiener, K.M., Wang, C.C., 1999. A *Trypanosoma brucei* bloodstream form mutant deficient in ornithine decarboxylase can protect against wild-type infection in mice. Exp. Parasitol. 91, 176–184.

Navas, I.M., Garcia-Fernández, A.J., Johnson, R.A., Reguera, R.M., Balaña-Fouce, R., Ordóñez, D., 1996. Structural determinants of putrescine uptake inhibition produced by cationic diamidines in the model of trypanosomatid *Crithidia fasciculata*. Biol. Chem. 377, 833–836.

Neal, R.A., Iwobi, M.V., Robert-Gero, M., 1989. Antileishmanial effect of free and encapsulated sinefungin against *Leishmania donovani* infections in BALB/c mice. C. R. Acad. Sci., Ser. III 308, 485–488.

Nishimura, K., Araki, N., Ohnishi, Y., Kozaki, S., 2001. Effects of dietary polyamine deficiency on *Trypanosoma gambiense* infection in rats. Exp. Parasitol. 97, 95–101.

North, M.J., Lockwood, B.C., Bremner, A.F., Coombs, G.H., 1986. Polyamine biosynthesis in trichomonads. Mol. Biochem. Parasitol. 19, 241–249.

Parry, L., Balaña-Fouce, R., Pegg, A.E., 1995. Post-transcriptional regulation of the content of spermidine/spermine *N*1-acetyltransferase by *N*1*N*12-bis(ethyl)spermine. Biochem. J. 305, 451–458.

Pegg, A.E., Wechter, R., Pakala, R., Bergeron, R.J., 1989. Effect of *N*1,*N*12-bis(ethyl)spermine and related compounds on growth and polyamine acetylation, content, and excretion in human colon tumor cells. J. Biol. Chem. 264, 11744–11749.

Pereira, C.A., Alonso, G.D., Paveto, M.C., Flawia, M.M., Torres, H.N., 1999. L-arginine uptake and L-phosphoarginine synthesis in *Trypanosoma cruzi*. J. Eukaryot. Microbiol. 46, 566–570.

Pérez-Pertejo, Y., Diaz-González, R., García-Estrada, C., Reguera, R.M., Balaña-Fouce, R., 2003. *S*-adenosylmethionine metabolism in Leishmania parasites. Recent Res. Dev. Microbiol. 7, 547–560.

Pérez-Pertejo, Y., Reguera, R.M., Balaña-Fouce, R., Ordóñez, D., in press. Characterization of a methionine adenosyltransferase over-expressing *Leishmania donovani* strain. Biochem. J.

Persson, L., Aslund, L., Grahn, B., Hanke, J., Heby, O., 1998. *Trypanosoma cruzi* has not lost its *S*-adenosylmethionine decarboxylase: characterization of the gene and the encoded enzyme. Biochem. J. 333, 527–537.

Persson, L., Jeppsson, A., Nasizadeh, S., 2004. Turnover of trypanosomal ornithine decarboxylases. Biochem. Soc. Trans. 31, 411–414.

Phelouzat, M.A., Lawrence, F., Robert-Gero, M., 1993. Characterization of sinefungin-resistant *Leishmania donovani* promastigotes. Parasitol. Res. 79, 683–689.

Phillips, M.A., Coffino, P., Wang, C.C., 1987. Cloning and sequencing of the ornithine decarboxylase gene from *Trypanosoma brucei*. Implications for enzyme turnover and selective difluoromethylornithine inhibition. J. Biol. Chem. 262, 8721–8727.

Reguera, R., Balaña-Fouce, R., Cubría, J.C., Alvarez, M.L., Ordóñez, D., 1994. Putrescine uptake inhibition by aromatic diamidines in *Leishmania infantum* promastigotes. Biochem. Pharmacol. 47, 1859–1866.

Reguera, R., Balaña-Fouce, R., Cubría, J.C., Alvarez, M.L., Ordóñez, D., 1995. Fluorinated analogues of L-ornithine are powerful inhibitors of ornithine decarboxylase and cell growth of *Leishmania infantum* promastigotes. Life Sci. 56, 223–230.

Reguera, R.M., Balaña-Fouce, R., Pérez-Pertejo, Y., Fernández, F.J., García-Estrada, C., Cubría, J.C., Ordóñez, C., Ordóñez, D., 2002. Cloning expression and characterization of methionine adenosyltransferase in *Leishmania infantum* promastigotes. J. Biol. Chem. 277, 3158–3167.

Roberts, S.C., Jiang, Y., Jardim, A., Carter, N.S., Heby, O., Ullman, B., 2001. Genetic analysis of spermidine synthase from *Leishmania donovani*. Mol. Biochem. Parasitol. 115, 217–226.

Roberts, S.C., Scott, J., Gasteier, J.E., Jiang, Y., Brooks, B., Jardim, A., Carter, N.S., Heby, O., Ullman, B., 2002. *S*-adenosylmethionine decarboxylase from *Leishmania donovani*. Molecular, genetic and biochemical characterization of null mutants and overproducers. J. Biol. Chem. 277, 5902–5909.

Roberts, S.C., Tancer, M., Jiang, J., Heby, O., Ullman, B., 2002. Polyamine metabolism in *Leishmania*. Abstracts of the XIII molecular parasitology meeting. Woods Hole.

Roberts, S.C., Tancer, M.J., Polinsky, M.R., Gibson, K.M., Heby, O., Ullman, B., 2004. Arginase plays a pivotal role in polyamine precursor metabolism in *Leishmania*. Characterization of gene deletion mutants. J. Biol. Chem. 279, 23668–23678.

Sakata, K., Fukuchi-Shimogori, T., Kashiwagi, K., Igarashi, K., 1997. Identification of regulatory region of antizyme necessary for the negative regulation of polyamine transport. Biochem. Biophys. Res. Commun. 238, 415–419.

Sakata, K., Kashiwagi, K., Igarashi, K., 2000. Properties of a polyamine transporter regulated by antizyme. Biochem. J. 347, 297–303.

Sánchez, C.P., González, N.S., Algranati, I.D., 1989. Stable ornithine decarboxylase in promastigotes of *Leishmania mexicana mexicana*. Biochem. Biophys. Res. Commun. 161, 754–761.

Satriano, J., Isome, M., Casero Jr., R.A., Thomson, S.C., Blantz, R.C., 2001. Polyamine transport system mediates agmatine transport in mammalian cells. Am. J. Physiol., Cell. Physiol. 281, C329–C334.

Schwarcz de Tarlovsky, M.N., Hernández, S.M., Bedoya, A.M., Lammel, E.M., Isola, E.L., 1993. Polyamines in *Trypanosoma cruzi*. Biochem. Mol. Biol. Int. 30, 547–558.

Seiler, N., 1987. Functions of polyamine acetylation. Can. J. Physiol. Pharm. 65, 2024–2035.

Seiler, N., Dezeure, F., 1990. Polyamine transport in mammalian cells. Int. J. Biochem. 22, 211–218.

Seiler, N., Delcros, J.G., Moulinoux, J.P., 1996. Polyamine transport in mammalian cells. An update. Int. J. Biochem. Cell. Biol. 28, 843–861.

Singh, S., Puri, S.K., Singh, S.K., Srivastava, R., Gupta, R.C., Pandey, V.C., 1997. Characterization of simian malarial parasite (*Plasmodium knowlesi*)-induced putrescine transport in rhesus monkey erythrocytes. A novel putrescine conjugate arrests in vitro growth of simian malarial parasite (*Plasmodium knowlesi*) and cures multidrug resistant murine malaria (*Plasmodium yoelii*) infection in vivo. J. Biol. Chem. 272, 13506–13511.

Svensson, F., Ceriani, C., Wallstrom, E.L., Kockum, I., Algranati, I.D., Heby, O., Persson, L., 1997. Cloning of a trypanosomatid gene coding for an ornithine decarboxylase that is metabolically unstable even though it lacks the C-terminal degradation domain. Proc. Natl. Acad. Sci. U. S. A. 94, 397–402.

Teixeira, S.M., DaRocha, W.D., 2003. Control of gene expression and genetic manipulation in the Trypanosomatidae. Genet. Mol. Res. 2, 148–158.

Tekwani, B.L., Bacchi, C.J., Pegg, A.E., 1992. Putrescine activated *S*-adenosylmethionine decarboxylase from *Trypanosoma brucei brucei*. Mol. Cell. Biochem. 117, 53–61.

Tomitori, H., Kashiwagi, K., Sakata, K., Kakinuma, Y., Igarashi, K., 1999. Identification of a gene for a polyamine transport protein in yeast. J. Biol. Chem. 274, 3265–3267.

Tomitori, H., Kashiwagi, K., Asakawa, T., Kakinuma, Y., Michael, A.J., Igarashi, K., 2001. Multiple polyamine transport systems on the vacuolar membrane in yeast. Biochem. J. 353, 681–688.

Vargiu, C., Cabella, C., Belliardo, S., Cravanzola, C., Grillo, M.A., Colombatto, S., 1999. Agmatine modulates polyamine content in hepatocytes by inducing spermidine/spermine acetyltransferase. Eur. J. Biochem. 259, 933–938.

Wallace, H.M., 1996. Polyamines in human health. Proc. Nutr. Soc. 55, 419–431.

Wallace, H.M., Fraser, A.V., Hughes, A., 2003. A perspective of polyamine metabolism. Biochem. J. 376, 1–14.

Weeks, R.S., Vanderwerf, S.M., Carlson, C.L., Burns, M.R., O'Day, C.L., Cai, F., Devens, B.H., Webb, H.K., 2000. Novel lysine-spermine conjugate inhibits polyamine transport and inhibits cell growth when given with DFMO. Exp. Cell. Res. 261, 293–302.

Wrenger, C., Luersen, K., Krause, T., Muller, S., Walter, R.D., 2001. The *Plasmodium falciparum* bifunctional ornithine decarboxylase, *S*-adenosyl-L-methionine decarboxylase, enables a well balanced polyamine synthesis without domain–domain interaction. J. Biol. Chem. 276, 29651–29656.

Xie, X., Gillies, R.J., Gerner, E.W., 1997. Characterization of a diamine exporter in Chinese hamster ovary cells and identification of specific polyamine substrates. J. Biol. Chem. 272, 20484–20489.

Yakubu, M.A., Basso, B., Kierszenbaum, F., 1992. DL-alpha-difluoromethylarginine inhibits intracellular *Trypanosoma cruzi* multiplication by affecting cell division but not trypomastigote–amastigote transformation. J. Parasitol. 78, 414–419.

Yarlett, N., Bacchi, C.J., 1988. Effect of DL-alpha-difluoromethylornithine on polyamine synthesis and interconversion in *Trichomonas vaginalis* grown in a semi-defined medium. Mol. Biochem. Parasitol. 31, 1–9.

Yarlett, N., Bacchi, C.J., 1994. Parasite polyamine metabolism: targets for chemotherapy. Biochem. Soc. Trans. 22, 875–879.

Yarlett, N., Martínez, M.P., Goldberg, B., Kramer, D.L., Porter, C.W., 2000. Dependence of *Trichomonas vaginalis* upon polyamine backconversion. Microbiology 146, 2715–2722.

Zou, Y., Wu, Z., Sirisoma, N., Woster, P.M., Casero Jr., R.A., Weiss, L.M., Rattendi, D., Lane, S., Bacchi, C.J., 2001. Novel alkylpolyamine analogues that possess both antitrypanosomal and antimicrosporidial activity. Bioorg. Med. Chem. Lett. 18, 1613–1617.